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THE CELL CONTENT OF MILK.*

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During the past few years the cell content of milk has received considerable attention. Those who first discovered the fact that milk contained cells supposed these cells were leukocytes. Whenever there is inflammation or suppuration there are always large numbers of pus cells, and according to Bergey a pus cell is simply a dead leukocyte. For the reason that leukocytes are found under inflammatory conditions it was supposed that the presence of these cells in milk indicated an inflammatory condition of the udder. While most investigators agree that some of the cells found in milk are leukocytes it is by no means certain that some of them are not epithelial cells. It was supposed that the number of cells found in milk was of prime importance in determining the presence or absence of inflammation.

There are two chief methods which are usually employed to enumerate the cells in milk. One of these is called the smeared-sediment method and the other is called the Doane-Buckley, or volumetric, method.

The smeared-sediment method was devised by Stokes of the Baltimore Board of Health, and was later modified by Stewart of the Philadelphia Board of Health, and Slack of the Boston Board of Health.

Briefly described, the smeared-sediment method consists in centrifugalizing one or two cubic centimeters of milk. The containers of the milk are small glass tubes closed at each end with a rubber stopper and the centrifuge is run at a high rate of speed. The sediment obtained is spread over a definite area on a cover glass, stained, and the number of cells counted.

The volumetric method was adapted by Charles F. Doane and S. S. Buckley at the time, respectively, dairyman and veterinarian at Maryland Experiment Station. The comparative results

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obtained by the smeared-sediment and volumetric methods have been studied by Doane, Russell and Hoffman, of Wisconsin, and Ward, of California. All are unanimous in pronouncing the volumetric method the most accurate one.

In the study of the cell content of milk we used only the volumetric method somewhat modified. The work done may be particularly divided into two parts: first, a study of the volumetric method of enumerating cells in milk; and second, a count of the cell content of milk in different cows and under different conditions for various lengths of time.

VOLUMETRIC METHOD OF ENUMERATING CELLS IN MILK.

Since this method was considerably modified we will describe it as it was used. Ten c.c. of milk were centrifugalized for 10 minutes in a graduated glass tube after heating to a temperature of 100° F. to 120° F. The heating was accomplished by immersing the tubes in warm water. A direct flame is liable to crack the tubes. Care should be taken not to heat the milk highly enough to precipitate the albumen, which would obscure the lines in the counting chamber. The centrifuge had a diameter of 13 in. when the cups were extended and ran at a speed of 2,000 revolutions per minute. With a machine of this diameter it was found impractical to run at a speed of less than 1,800 revolutions per minute. This speed was necessary not only to throw down the cells but to remove all of the fat. After centrifugalizing for 10 minutes the fat was removed by means of a cotton swab. The tubes were then centrifugalized for one minute more and the remaining fat was removed. In whirling the tubes a second time, care should be taken to have the liquid at the same level in each tube, as otherwise the machine will be unbalanced. All of the liquid possible was then withdrawn. Care should be taken not to disturb the sediment in the bottom of the tubes and for this reason it is impossible to withdraw all of the liquid. In most cases it was withdrawn to the 0.5 c.c. mark on the tube. In withdrawing this liquid we used a bent pipette the intake of which was on the side of the tube instead of at the end and this opening was about a quarter of an inch from the end of the tube. The bent pipette was convenient because it allowed the operator to hold the tube in such a position that it could readily be seen when all of the liquid necessary had been removed.

After the supernatant liquid was withdrawn a stain was added. The stain was made by mixing 15 drops of a saturated gentian-violet solution with 100 c.c. of a 0.6 per cent solution of sodium chloride. This solution tinted the cells a faint purple and yet did not stain everything such a deep color as to make some of the objects in the counting chamber unrecognizable. Some stains did have this objectionable feature. Russell and Hoffman used no stain but we found this proved rather hard on the eyes, and in some cases it was difficult to identify objects under the microscope unless the stain was used. A good example of such an object would be a piece of casein with a fat globule entangled in it. The sediment is usually packed so hard in the bottom of the tube that a platinum needle or some similar instrument is necessary to break up the sediment. Enough of the stain was added to make the volume of liquid up to one c.c., which made it an easy basis of calculation. After the stain was thoroughly mixed with the sediment a drop of the mixture was transferred to the counting chamber and the cells enumerated. The drop of liquid in the chamber was rejected and a new one taken under the following conditions:

1. If the cover glass did not fit snugly against the slide. This would be due to foreign material underneath the cover glass.
2. If the drop did not cover the entire ruled area.
3. If the liquid overflowed the counting chamber and ran between the cover glass and the slide.
4. If on examination the cells were not fairly evenly distributed over the entire field.
5. If any air bubbles were under the cover glass.
6. If any foreign material was found in the chamber which would cover up the cells. This might include precipitated casein, particles of skin from the udder of the cow, and fat globules.

In using the counting chamber it is best to have some definite method of enumerating cells and always use that method. This will save much useless confusion. For instance, we always began at the upper left-hand corner of the counting chamber and worked downward, counting the four blocks of $\frac{1}{16}$ sq. mm. each. We then began at the bottom and counted upward on the squares at the extreme right hand of the ruled area. The next four squares were counted working downward, and then beginning again at the bottom, the four remaining squares were counted. There is no particular reason for this method except that it is a fixed one, and by setting down the number of cells in the order in which they were counted one could tell at a glance how many cells were in any particular square without enumerating them again.

METHOD OF COMPUTING RESULTS.

The results obtained were expressed in terms of cubic centimeters. We will suppose, for example, that there were in the entire field 55 cells. Since the chamber is 0.1 mm. deep the 55 was multiplied by 10, making 550 cells per c.mm. There are, however, 1,000 c.mm. in a c.c., so that to reduce our results to c.c. the 550 was multiplied by 1,000, making 550,000 cells. However, we started with 10 c.c. of milk and counted and concentrated in one c.c. of milk all of the cells there were in 10 c.c. The results are, therefore, 10 times too large, and dividing 550,000 by 10 we get a result of 55,000 cells per c.c.

The practice of counting part of a field and then estimating the number of cells in the entire chamber is sometimes followed. Since the counting chamber contains 16 squares, either four or eight squares are usually counted and the results then multiplied by four or two respectively. In order to see how accurate such an estimate would be, 100 samples were counted in which a comparison of counting four squares, eight squares, and the entire field was made. When four squares were counted and an estimate made there was an average variation of 8.89 per cent. When eight squares were counted and an estimate made there was an average variation of 6.57 per cent. A count of the entire field was of course taken as a basis for computing the percentage of variation.

In order to test the accuracy of the volumetric method, two counts each were made of 56 samples of milk. There was found to be an average variation of 12.76 per cent between the two counts. This is considerably higher than the results obtained by

Russell and Hoffman, who found a variation of only 5.6 per cent. There were, however, included in these counts three or four abnormally high variations. One variation was over 55 per cent. While these variations were abnormal and greatly increased the average variation, it was thought best to include them because they show the variations which it is possible to obtain by using this method.

In separating milk with a centrifugal separator the milk is heated before separation in order to make the removal of the fat more thorough. The presence of fat globules in the counting chamber is a disturbing factor and it was thought only reasonable that warming the milk before centrifugalizing would help to remove the fat more thoroughly. A trial proved this to be the case, but also showed that milk which was warmed before centrifugalizing gave a much higher cell count than milk which was not warmed. This was probably due to the fact that warming milk expanded it and therefore made it easier to throw the cells to the bottom of the tube. Two counts each of 50 samples were made, one from a heated sample and one from an unheated sample. The unheated samples were centrifugalized at a temperature of from 60° to 70° F. and the temperature of the heated samples was 100° to 120° F. The samples which were heated before centrifugalized gave an increase in cell content of 13.6 per cent. Care should be taken not to heat the samples hot enough to precipitate the albumen, as this would obscure the ruled area in the counting chamber.

VARIATIONS IN MILK FROM DIFFERENT COWS.

In all of the samples examined, no milk was found free from cells. In an examination of the milk from 50 different cows the cell content varied from 4,000 to 3,576,000. To all appearances both cows from which these samples were taken were perfectly normal and healthy, and there was no apparent reason for the difference in count.

COMPARATIVE COUNTS OF FORE MILK, MIDDLE MILK, AND LAST MILK.

In this experiment, the first 10 or 15 streams from each teat were regarded as the fore milk. The middle milk was obtained when the cow was approximately half milked, and the last milk consisted of what is ordinarily termed the "strippings." Fifteen different samples were compared and in every case the last milk contained the greatest number of cells per c.c. In 11 samples of the 15 counted, the first milk gave the smallest count, the middle milk next highest, and the last milk highest. The percentage increase in cell content per c.c. of the last milk over the fore milk was 53.85, and that of the last milk over the middle milk was 52.14.

EFFECT OF MANIPULATION OF THE UDDER.

It is not definitely known whether the cells found in milk are true leukocytes or whether they are epithelial cells. In either case it seemed reasonable to suppose that manipulation of the udder would tend to increase them. The manipulation would break down cells and if vigorous enough to produce inflammation would cause an increase in leukocytes.

The method of conducting this experiment was as follows: A count of the milk was taken at night and on the following morning. On the following evening, 24 hours after the first count, the udder was vigorously manipulated for five minutes. The cow was then milked and a count of the milk made.

Generally speaking, the results of this experiment were unsatisfactory. In the majority of cases there was an increase in cell content after the udder was manipulated, but this increase was sometimes so small that it was uncertain whether the increase was due to manipulation or some other causes. Nine experiments were made and the average percentage of increase was 11.98 for the first count and 35.29 for the second count. In a small percentage of the nine experiments there was a decrease in cell content per c.c. after the udder was manipulated.

VARIATIONS IN A LONG PERIOD.

In studying the cell content of milk for a long period of time three cows of different breeds were selected. Golden Daisy was a Jersey and freshened October 6, 1908. Sigma was a Holstein and freshened October 8, 1908. Lady Benton was a Shorthorn and freshened October 10, 1908. The milk from the first two was examined for a period of a little over seven months. Lady Benton was sold for beef before the experiment was concluded and her milk was examined for a period of about five months only.

Several items of interest were noted in the examination of the milk for this long period of time. In the first place, the cell content of the milk per c.c. varies both upward and downward, but the variation in individual cows was within certain limits. In the case of Golden Daisy, the cell content of the milk varied normally from 4,000 to 99,000, but was usually between 40,000 and 80,000. The milk from Sigma was not so uniform in cell count as was that from Golden Daisy, varying from 21,000 to 272,000; yet the count of the milk from Sigma was normally higher than that from Golden Daisy, and the milk from the former varied in cell content normally from about 40,000 to 150,000.

The milk from Lady Benton usually gave a very high count. The number of cells varied from 107,000 to 1,063,000. The count rarely fell much below 300,000 and was usually much higher.

The counts from these three cows present a wide variation for which we cannot account. It seems certain that this variation is not due to breed. Another Shorthorn cow gave a count of 18,000 cells per c.c. It was our general experience that the individual count varied without regard to breed.

During the two weeks while this experiment was in progress, Golden Daisy was ill of indigestion. During this time the amount of milk she gave was very small and the cell content per c.c. was abnormally high. One week the cow gave 25.5 pounds of milk and the second week 17.6 pounds of milk. The cell count for these respective weeks was 988,000 and 314,500 cells per c.c. The high cell content may perhaps be accounted for on the grounds of inflammation of the udder.

RELATION BETWEEN FAT CONTENT AND CELL CONTENT OF MILK.

It was suggested that cows giving milk with a high percentage of fat might yield milk of a higher cell content than those giving milk with a low percentage of fat. It is fairly well known that in the process of milk secretion the cells of the acini are very active and are rapidly disintegrated; it therefore seemed reasonable that the more fat in milk, the greater number of cells there would be.

On investigation this theory did not hold good. The average percentage of fat found in the evening milk of Golden Daisy was 6.05 and the average cell content of the milk per c.c. was 82,214.

The average percentage of fat found in the evening milk of Sigma was 3.7 and the average cell content of the milk per c.c. was 118,681. The milk from Sigma averaged 2.35 per cent less fat than did the milk from Golden Daisy; yet the milk from Sigma contained 36,467 more cells per c.c. than the milk from Golden Daisy. It was also found that the milk from Lady Benton contained less fat than the milk from Golden Daisy, yet the milk of the former had a higher cell content than that of the latter.

On the other hand the results did not show that a low percentage of fat necessarily indicated a high cell content. Sigma gave milk lower in fat than did Lady Benton and yet her milk had a lower cell content.

Results obtained from the examination of milk from other cows were the same as in the case of the three cows mentioned above.

In order to test this point still further, those tests which were above the average percentage of fat were themselves averaged. Then the cell counts corresponding to the percentages of fat above the average were averaged. The results obtained indicated that the cell content had no relation to a high percentage of fat.

It is possible, however, that the percentage of fat present in the milk and the number of separate globules which go to make up that percentage have no definite relation. It is well known that the fat globules in milk from different breeds of cows vary in size. It is reasonable to suppose that even though the percentage of fat in milk was low, the number of fat globules might be large, and in consequence the cell content might be high.

RELATION OF NUMBER OF CELLS TO QUANTITY OF MILK PRODUCED.

It has been pointed out already that under normal conditions the cell content of milk per c.c. from an individual cow is fairly constant. By comparing the cell count of the milk with the number of pounds given, we are led to conclude that the total number of cells decreases as the quantity of milk decreases.

During the week of October 18, 1908, Golden Daisy gave 195.6

pounds of milk; during the week of April 19, 1909, she gave 83.7 pounds of milk. During the week of October 13, 1908, Sigma gave 263.8 pounds of milk, and during the week of April 19, 1909, 203.6 pounds of milk. During the week of October 14, 1908, Lady Benton gave 156.9 pounds of milk, and during the week of February 16, 1909, she gave only 21.6 pounds of milk. In each case 10 c.c. of the milk were examined, and yet we found that the number of cells per c.c. obtained at the last count was neither abnormally high nor abnormally low. Unless the number of cells decreased as the milk decreased, we would get an abnormally high number of cells per c.c. in the last count.

COMPARISON OF COLOSTRUM AND NORMAL MILK.

In the following table is given a comparison of the cell content of colostrum with the cell content of milk from the same cow later in the lactation period. The table needs no special explanation. It will be seen that the cell content of colostrum was always higher than was the normal milk later in the lactation period. This is what would naturally be expected because of the sudden activity of the udder after a period of inactivity. Apparently most of the cells present in colostrum are broken-down epithelial cells.

TABLE SHOWING A COMPARISON OF THE CELL CONTENT OF COLOSTRUM WITH THAT OF NORMAL MILK.

Sample Number	Colostrum Cells per c.c.	Number of Milking	Normal Milk Cells per c.c.
1	713,500	8th	18,000
2	3,935,000	7th	13,000
3	332,000	3d	20,000
4	500,000	6th	68,000
5	553,000	5th	49,000
6	332,000	21st	20,000

EFFECT OF AN INJURED QUARTER OF THE UDDER.

In the midst of our investigations of this subject, one of the cows in the herd suddenly began to give milk that was decidedly abnormal. An examination showed that one quarter of the udder was affected. Thereafter the milk from this quarter was drawn in a vessel separate from the milk of the other three quarters. A cell count was made of the two samples with the following results:

Infected quarter, 62,400,000 cells per c.c.
Three sound quarters, 407,000 cells per c.c.

These two counts are interesting because they show that one quarter may be seriously affected without similarly affecting the other three quarters.

LEUKOCYTE STANDARDS.

There has been much recent discussion regarding the establishment of a standard for the cell content of milk, called a leukocyte standard. It has been pointed out that in many cases it is almost impossible to distinguish between leukocytes and the other cells. Some investigators have suggested that the maximum number of cells allowed should be 100,000 per c.c. From the results obtained in this investigation, it would seem unwise to set any standards for the cell content of milk until we have more definite information as to causes and effects. If the standard of 100,000 cells per c.c. were enforced, the milk from two of the three cows here considered would have to be rejected; yet these animals were, so far as we were able to learn, perfectly normal and healthy. It has been shown that the total number of cells seems to decrease as the quantity of the milk decreases. This would indicate that the formation of cells, or leukocytes, is a normal function of milk production.